

## **REMARKS**

In response to the Office Action mailed October 22, 2002, Applicants have amended the claims, which when considered with the following remarks, is deemed to place the present application in condition for allowance. Claims 12, 13, 15, 16, 18, 19, 20, 22, 23, 28, 29, 31, 42, 46 and 51 have been cancelled without prejudice. Favorable consideration of all pending claims is respectfully requested.

In the first instance, Applicants through the undersigned, thank Examiners Collins and Bui for helpful comments provided during the course of a telephone interview on Monday, March 17, 2003. The Examiners indicated that the presently amended claims would be favorably considered.

In the Office Action Of October 22, 2002, the Examiner has indicated that the amendments to the specification have not been entered because a marked up version was not included in Applicants' response filed August 13, 2002. The present response to the Office Action of October 22, 2002 includes the same amendments to the specification submitted previously by Applicants and in addition, includes a marked up version. Entry of the amendments to the specification is respectfully requested.

Claims 53 and 54 remain objected to under 37 C.F.R. §1.75(c) as allegedly being in improper form because a multiple dependent claim should refer to other claims in the alternative only. As presently amended, claims 53 and 54 depend from claims 2, 5, 7-11, 13-25, 27 *or* 31, rather than claims 2, 5, 7-11, 13-25, 27 and 31. Withdrawal of the objection under 37 C.F.R. § 1.75 is therefore respectfully requested.

Claims 2, 5, 7-25, 27-31, 36-51 and 55-57 remain rejected under 35 U.S.C. §112, first paragraph, as allegedly violative of the written description requirement. According to the Examiner, recitation that the cyclin-dependent kinase inhibitor (CKI) interacts with CDC2a does

not overcome the written description rejection, as a correlation between a particular structural domain of a CKI and the interaction with CDC2a is not known or disclosed. Also according to the Examiner, a correlation between a particular structural domain of a CKI and the function of altering growth characteristics in a plant is also not known or disclosed. The Examiner furthermore states that the cited reference of Nakayama et al. (1998) *BioEssays* 20: 1020-1029, is relevant to the description of the instant invention insofar as Nakayama et al. teach that cyclin-dependent kinase inhibitors may exhibit structural and functional differences as well as similarities, such that one must describe in some manner the structural domains of CKIs that are required to achieve a desired effect.

Applicants have amended the claims to recite a cyclin dependent kinase inhibitor (CKI) which "binds CdC2a" rather than "interacts with CdC2a." Applicants traverse the rejection and respectfully submit the following. The prior art does contain teaching about the structure-function relationship in CKIs. Wang et al. (The Plant Journal 15: 501-510, 1998), a copy of which is submitted as Exhibit A, have shown that N-terminal deletions in ICK1 do not decrease ICK1-CDC2a binding. C-terminal fragments of ICK1 starting at amino acid 154 onwards, were still able to interact with CDC2a (see Figure 6). In addition, a study by Lui et al. (The Plant Journal 21 (4): 379-385; 2000), a copy of which is submitted herewith as Exhibit B, discloses the binding properties of deletion fragments of 2 CKI proteins (CKI1 & CKI2) to the cyclin dependent kinases CDC2a and CDC2b. Table 1 in this paper shows various fragments of ICK2 which do or do not interact with the CDK enzymes when using a 2-hybrid screen in yeast. From this table, it is evident that the C-terminal part of the protein is responsible for the binding to CDC2a. An alignment of CKI amino acid sequences reveals that the carboxy-terminal part of the proteins is conserved. It is also this C-terminal part of the plant proteins that bares the closest homology to the animal cyclin-dependent kinase inhibitor, p27<sup>Kip1</sup>, as shown in Figure 1(c) of

Lui et al. Furthermore, the inventors of the present application have shown that other CKI proteins also share this conserved C-terminus, as is depicted in Figure 12 of the application for 8 different CKI proteins.

In addition, submitted herewith as Exhibit C, is further experimental data showing *Arabidopsis* plants overexpressing two CKI proteins (KRP6 and KRP7 disclosed in the present application), and having the same phenotypic characteristics observed for CKI2 (also known as ICK2 or KRP2) overexpressing plants as disclosed in the present application. Furthermore, the study by Zhou et al. (Plant Cell Reports 20: 967-975; 2002)(submitted as Exhibit B with Applicants' prior amendment dated August 9, 2002) discloses that overexpression of three CKI proteins (ICK1, ICK4 and ICKCr) in *Arabidopsis* resulted in the same plant phenotype as already described in Fig. 3 for CK12 of the present application. Thus, Applicants respectfully submit that plant CKI proteins are different from their animal counterparts, an opinion that was also expressed by Wang et al. (1998, see abstract), and that the observations made by Nakayama and Nakayama cannot be extrapolated to plant cyclin-dependent kinase inhibitors. Withdrawal of the rejection of claims 2, 5, 7-25, 27-31, 36-51 and 55-57 is therefore warranted.

Claims 2, 5, 7-25, 27-31 and 36-57 remain rejected under 35 U.S.C. § 112, first paragraph, as allegedly directed to non-enabled subject matter. According to the Examiner, the specification, while being enabling for a method of decreasing cyclin-dependent kinase activity in *Arabidopsis* plants which comprises introducing into a plant a nucleotide sequence of SEQ ID NO 1 encoding the homologous cyclin-dependent kinase inhibitor ICK2 of SEQ ID NO 2, wherein said method increases the level of ICK2 in a cell, increases plant cell size in petals, leaves and stems, decreases cell number in a plant, increases leaf serration, increases the size of the stomata, reduces petal size, reduces leaf venation, decreases endoreduplication and ploidy levels in mature leaf cells, and reduces seed size, compared to wild type plants, does not

reasonably provide enablement for methods of altering growth characteristics in plants which comprise introducing into a plant a nucleotide sequence encoding a cyclin-dependent kinase inhibitor, wherein said method modifies plant cell size, modifies cell number in a plant, alters leaf shape, alters leaf size, increases gas exchange and photosynthesis, alters tissue or organ shape or size, alters leaf venation, facilitates the transition from the mitotic cycle to G1 arrest, alters seed size, or alters seed shape, compared to wild type plants. The Examiner maintains that the use of the CaMV 35S promoter as exemplified does not enable the invention for the use of a CKI to alter any plant growth characteristic in any way.

In response to the rejection, and in order to advance prosecution of this application, Claims 2, 5, 7-25, 27-31 and 36-57 have been amended to recite a method of decreasing cyclin-dependent kinase activity in plants (claim 2), a method for increasing the level of CKI in a cell (claim 5), a method of increasing plant cell size (claim 7), a method for decreasing cell number in a plant (claim 11), a method for altering leaf serration (claim 14), a method for increasing the size of the stomata (claim 17), a method for reducing petal size (claim 21), a method for reducing leaf venation (claim 25), a method for decreasing endoreduplication and ploidy levels in leaf cells (claim 27), and a method for reducing seed size (claim 30), compared to wild type plants. In all of these amended claims, the method entails introducing into a plant cell a nucleic acid molecule encoding a cyclin dependent kinase inhibitor (CKI) which binds CDC2a. Applicants have shown the same phenotypic characteristics with respect to leaf shape and morphology using three different CKI genes, CK12, KR6 and KRP7, the three genes disclosed in the present application. Moreover, Zhou et al. (2002) have shown the same phenotypic characteristics upon overexpression of three additional, distinct CKI genes; ICK1, ICK2, and ICKCr. In view of the amendments to the claims, and the remarks hereinabove, withdrawal of the rejection of claims 2,

5, 7-25, 27-31 and 36-57 under the enablement provision of 35 U.S.C. §112, first paragraph, is respectfully requested.

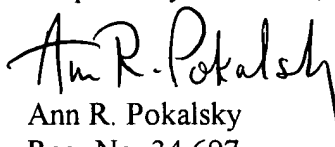
Claim 36 remains rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite in the recitation of "derived thereof". As presently amended, claim 36 recites "obtained" rather than "derived". Claim 36 is also rejected under 35 U.S.C. 112, second paragraph, as allegedly indefinite in its recitation of "essentially the same characteristics". In order to advance prosecution on this application, claim 36 has been amended to recite "essentially the same characteristics resulting from the transgene".

Withdrawal of the rejection of claim 36 under 35 U.S.C. §112, second paragraph

Claims 2, 5, 7, 11, 14-15, 17-19, 21, 25, 27, 30-31, 36, 56 and 57 have been rejected under 35 U.S.C. 112, second paragraph, as allegedly indefinite in the recitation of "interacts with CDC2a". As presently amended, claims 2, 5, 7, 11, 14, 17, 21, 25, 27, 30, 36, 56 and 57 recite or depend from claims which recite "binds CDC2a." Withdrawal of the rejection of claims 2, 5, 7, 11, 14-15, 17-19, 21, 25, 27, 30-31, 36, 56 and 57 under 35 U.S.C. §112, second paragraph, is therefore respectfully requested.

In view of the forgoing amendments and remarks hereinabove, it is respectfully submitted that the present claims are in condition for allowance, which action is respectfully requested.

Respectfully submitted,



Ann R. Pokalsky  
Reg. No. 34,697  
Attorney for Applicants

DILWORTH & BARRESE  
333 Earle Ovington Blvd.  
Uniondale, NY 11553  
(516) 228-8484  
ARP:bg

## **VERSION WITH MARKINGS TO SHOW CHANGES MADE**

### **IN THE SPECIFICATION:**

#### **Page 9, lines 19-20:**

Figure 8A is a [otomicrograph] photomicrograph of stomata in [the] abaxial epidermis of a leaf of an *A. thaliana* Col-O control plant.

#### **Page 10, lines 7-8:**

Figure 11 is a Western blot showing CKI2,-CDC2aAt and Rubisco protein levels and CDK kinase activity. Total soluble protein was extracted from leaves of one wild-type Col-O line (lane 1) and four independent CKI2 transgenic lines (lanes 2 through 5). Protein samples were analyzed by Western blotting for the visualization of CKI2 protein and CDC2aAt protein. Rubisco was used as a marker for equal protein loading. CDK kinase activity was measured using p10<sup>Cks1At</sup> Sepharose beads and Histone H1 as substrate.

#### **Page 104, lines 25-32:**

The level of ICK2 mRNA and protein in the transgenic plants exceeded the amount found in untransformed plants as shown in Figure 11 for the ICK2 (CKI2) protein. Concurrently the amount of Cdc2a protein is increased and the presence of ICK2 protein correlated with a moderate decrease in extractable CDK activity [(Figure 11)] Figure 10.

[Presence of the ICK2 protein correlated with a moderate decrease in extractable CDK activity (Fig.)]

#### **Page 108, last paragraph:**

CKI2 expressing plants produce smaller seeds than wild type plants. The shape of the seed is also affected. See e.g., Figures [8] 9A and [8] 9B.

**IN THE CLAIMS:**

2. (Twice Amended) A method for [controlling or altering growth characteristics] decreasing cyclin-dependent kinase activity in a plant, comprising the steps of:

(i) introducing into a plant cell a nucleic molecule encoding a cyclin-dependent kinase inhibitor (CKI) which [interacts with] binds CDC2a, under the control of a regulatory sequence which controls expression of the cyclin-dependent kinase inhibitor;

(ii) expressing said nucleic acid molecule; and

(iii) regenerating a plant therefrom, which plant has [altered growth characteristics] decreased cyclin dependent kinase activity.

5. (Twice Amended) A method for increasing the level of cyclin-dependent kinase inhibitor (CKI) which [interacts with] binds CDC2a, in a plant cell relative to corresponding cells of a wild type plant, said method comprising the steps of:

(i) introducing into a plant cell a nucleic acid molecule encoding a cyclin-dependent kinase inhibitor under the control of a promoter which functions in plants; and

(ii) expressing said nucleic acid molecule in said plant cell, thereby increasing the level of cyclin-dependent kinase inhibitor in said plant cell.

7. (Amended) A method for [modifying] increasing plant cell size, said method comprising the steps of:

(i) introducing into a plant cell a nucleic acid molecule encoding a cyclin-dependent kinase inhibitor (CKI) which [interacts with] binds CDC2a, under the control of promoter which functions in plants; and

✓ (ii) expressing said nucleic acid molecule in said plant cell, thereby [modifying] increasing plant cell size.

11. (Twice Amended) A method for [modifying] decreasing cell number in a plant, comprising the steps of:

(i) introducing into a plant cell a nucleic acid molecule encoding a cyclin-dependent kinase inhibitor (CKI) which [interacts with] binds CDC2a, under the control of a promoter which functions in plants;

(ii) expressing said nucleic acid molecule in said plant cell; and

(iii) regenerating a plant from said plant cell, wherein said plant has [modified] decreased cell number.

14. (Amended) A method of [altering] increasing leaf serration [shape] in a plant, comprising the steps of:

(i) introducing into a plant cell a nucleic acid molecule encoding a cyclin-dependent kinase inhibitor (CKI) which [interacts with] binds CDC2a, under the control of a promoter which functions in plants;

(ii) expressing said nucleic acid molecule in said plant cell; and

(iii) regenerating a plant from said plant cell, said plant having [altered] increased leaf serration [shape].

17. (Twice Amended) A method of increasing stomata size of a plant, comprising the steps of:

(i) introducing into a plant cell a nucleic acid molecule encoding a cyclin-dependent kinase inhibitor (CKI) which [interacts with] binds CDC2a, under the control of a promoter which functions in plants; and

(ii) expressing said nucleic acid molecule in said plant cell; and

(iii) regenerating a plant from said plant cell, said plant having increased stomata size relative to corresponding wild type plants.



21. (Twice Amended) A method of [altering tissue or organ] reducing petal size in a plant, comprising the steps of:

- (i) introducing into a plant cell a nucleic acid molecule encoding a cyclin-dependent kinase inhibitor (CKI) which [interacts with] binds CDC2a, under the control of a promoter which functions in plants;
- (ii) expressing said nucleic acid molecule in the plant cell; and
- (iii) regenerating a plant from said plant cell, wherein said plant has flowers with [altered] reduced petal size.

25. (Twice Amended) A method of [altering] reducing leaf venation [pattern] in a plant [leaf], comprising the steps of:

- (i) introducing into a plant cell a nucleic acid molecule encoding a cyclin-dependent kinase inhibitor (CKI) which [interacts with] binds CDC2a, under the control of a promoter which functions in plants;
- (ii) expressing said nucleic acid molecule in the plant cell; and
- (iii) regenerating a plant from said plant cell, wherein said plant has leaves with reduced leaf [an altered] venation [pattern].

27. (Twice Amended) A method of [promoting the transition from the mitotic cycle to G1 arrest] decreasing endoreduplication and ploidy level in a plant cell, comprising the steps of:

- (i) introducing into a plant cell a nucleic acid molecule encoding a cyclin-dependent kinase inhibitor (CKI) which [interacts with] binds CDC2a, under the control of a promoter which functions in plants; and
- (ii) expressing said nucleic acid molecule in the plant cell.

30. (Twice Amended) A method of [altering] reducing plant seed size, comprising the steps of:

(i) introducing into a plant cell a nucleic acid molecule encoding a cyclin-dependent kinase inhibitor (CKI) which [interacts with] binds CDC2a, under the control of a promoter which functions in plants;

(ii) expressing said nucleic acid molecule in the plant cell; and

(iii) regenerating a plant from said plant cell, wherein said plant has decreased seed size relative to corresponding wild type plants.

36. (Twice Amended) A transgenic plant, a variety [derived thereof] obtained therefrom with essentially the same characteristics resulting from the transgene, a plant part, or plant cell which comprises a nucleotide sequence encoding a cyclin-dependent kinase inhibitor (CKI) which [interacts with] binds CDC2a, under the control of a promoter which functions in plants wherein said nucleotide sequence encoding a cyclin-dependent kinase inhibitor is heterologous to the genome of the transgenic plant, or is homologous but additional to the genome of the transgenic plant or has been introduced into the transgenic plant, plant part or plant cell by recombinant DNA means.

37. (Amended) The transgenic plant of claim 36 having [altered growth characteristics] decreased cyclin-dependent kinase activity.

38. (Amended) The transgenic plant of claim 36 having [altered leaf shape] an increased level of CKI.

43. (Amended) The transgenic plant of claim 36 having flowers with [altered] reduced petal size.

44. (Amended) The transgenic plant of claim 36 having [an altered venation pattern] reduced leaf venation.

45. (Amended) The transgenic plant of claim 36 having cells with [altered] decreased ploidy levels.

47. (Amended) The transgenic plant of claim 36 having [altered] reduced seed size.

49. (Twice Amended) The transgenic plant of claim 36, [comprising] wherein at least one of petals, leaves or stems comprise cells of increased size relative to corresponding wild type plants.

53. (Twice Amended) The method of claims 2, 5, 7-11, 13-25, 27, 30, [and] or 31, wherein the nucleic acid molecule comprises the nucleotide sequence as set forth in SEQ ID NO:1.

54. (Twice Amended) The method of claims 2, 5, 7-11, 13-25, 27, 30 [and] or 31 wherein the CKI comprises the consensus amino acid sequence as set forth in any one of SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38 or SEQ ID NO:39.

56. (Twice Amended) Harvestable parts or propagation material from the transgenic plant of claim 36, comprising the CKI which [interacts with] binds CDC2a that was introduced into the parent plant.

57. (Twice Amended) Cut flowers from the transgenic plant of claim 36, comprising the CKI which [interacts with] binds CDC2a that was introduced into the parent plant.

**Claims 12, 13, 15, 16, 18, 19, 20, 22, 23, 28, 29, 31, 42, 46, and 51 are cancelled without prejudice.**